

# scVelo

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An abbreviated version of this protocol was published in eLIFE in Nov 2020

Single nuclei RNA-seq of mouse placental labyrinth development

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## Detailed protocol

Detailed Protocol for scVelo for Single-nuclei RNA seq of the mouse placental labyrinth development

1. Single cell velocity trajectory analysis is performed in python using the Velocyto package (LaManno, 2018). Install velocity according to the instructions from the developers (<http://velocyto.org/velocyto.py/install/index.html>).
2. Generate intron/exon counts from expression matrices generated by 10x Genomics CellRanger3.0 using the run10x command in velocyto. This will generate loom files for each separate sample.
3. Concatenate these matrices into one file for all timepoints using the loompy package loompy.combine function in python. This can be done in jupyter notebooks.
4. To analyze the velocity for cells identified and clustered according to prior analyses performed in Seurat, convert the Seurat data to the loom format using the as.loom command in Seurat in R ([https://satijalab.org/seurat/articles/conversion\\_vignette.html](https://satijalab.org/seurat/articles/conversion_vignette.html)).
5. Install scvelo according to the instructions from the developers (<https://scvelo.readthedocs.io/installation.html>).
6. The scVelo analysis script is attached, and the steps are outlined below.
7. To filter the output loom from velocyto for only the cells used in the trophoblast analysis from Seurat, use the utils.merge function within scVelo. For our data, the concatenated output from velocity (AllStages\_velocity\_output.loom) and the integrated trophoblast dataset (AllStages\_TrophoblastNuclei.loom) are available for download here: [https://figshare.com/projects/Single\\_nuclei\\_RNA\\_seq\\_of\\_mouse\\_placental\\_labyrinth\\_development/92354](https://figshare.com/projects/Single_nuclei_RNA_seq_of_mouse_placental_labyrinth_development/92354).
8. Use the scvelo.pp.moments function to calculate the moments for velocity estimation, using the default settings of using 30 PCs and 30 neighbors. Then the proportion of spliced, unspliced, ambiguous reads was determined using scvelo.utils.show\_proportions.
9. The velocity was then calculated using scvelo.tl.velocity command, specifying the mode='stochastic'. The velocity graph was then computed using scv.tl.velocity\_graph with default parameters.
10. The data were then split by timepoint (E9.5, E10.5, E12.5, E14.5) and the velocity embeddings visualized using the scvelo.pl.velocity\_embedding function.
11. The magnitude of the velocity vectors was estimated from the velocity embeddings for each cell given by the 'velocity\_umap\_cell\_embeddings', by calculating the distance from the cell in umap space from the starting coordinate or each cell (0,0) to the coordinate of the velocity embedding provided by the 'velocity\_umap\_cell\_embeddings'. The units for this measurement are arbitrary and specific to the UMAP space.

## Related files

scvelo\_script.html.zip



**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Marsh, B. (2021). scVelo. Bio-protocol Preprint. [bio-protocol.org/prep935](https://bio-protocol.org/prep935).
2. Marsh, B. and Belloch, R.(2020). Single nuclei RNA-seq of mouse placental labyrinth development. eLIFE. DOI: [10.7554/eLife.60266](https://doi.org/10.7554/eLife.60266)

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